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(54) Title: THREE NEW NON-POLYGLUTAMATABLE DEAZAAMINOPTERINS AND PROCESS FOR PREPARING THE SAME

(57) Abstract

Three new non-polyglutamatable glutamic acid derivatives in the antifolate series are provided as well as procedures for their preparation. These compounds are: 4-amino-acid-4-deoxy-10-deazapteroyl-γ-methyleneglutamic acid, (1); 4-amino-4-deoxy-10-ethyl-10-deazapteroyl-γ-methyleneglutamic acid (2); and 4-amino-4-deoxy-10-deazapteroyl-3-hydroxyglutamic acid (3). None of the compounds undergo polyglutamylation as determined by their inability to serve as substrates for folylpolyglutamate synthetase (FPGS). Compounds (1) and (2) are transported more effectively to tumour cells and they are more powerful in inhibiting the growth of human leukaemia cells in culture as compared to the standard anticancer drug methotrexate. All three compounds (1, 2, and 3) inhibit human dihydrofolate reductase to the same extent as methotrexate.

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# THREE NEW NON-POLYGLUTAMATABLE DEAZAAMINOPTERINS AND PROCESS FOR PREPARING THE SAME

## Background of the Invention

### Field of the Invention

This invention relates to anticancer agents and the processes for their manufacture.

### Origin of Invention

The invention described herein was in part made in the course of work under a grant from the National Institutes of Health, Department of Health, Education and Welfare.

Cancer is an acute or chronic disease of man which is characterised by abnormal tissue growth and destruction that can be effectively treated with anticancer drugs such as methotrexate.

In Biochemical and Biophysical Research Communications 52:27 (1973), Baugh, Krumdieck and Nair reported on the metabolism of the well known anticancer drug methotrexate to its poly-$\gamma$-glutamates in human tissues. Nair and Baugh in Biochemistry 12:3923 (1973) reported the chemical synthesis of the poly-$\gamma$-glutamyl metabolites of methotrexate, the formation of these metabolites in rodent tissues, and the hydrolytic susceptibility of these metabolites to the enzyme "conjugase," derived from hog kidney and human plasma. Methotrexate polyglutamates are relevant to cancer chemotherapy because their formation is related to toxicity; they afflux from the cell at a slower rate than methotrexate and they are more inhibitory to thymidylate synthase and AICAR transformylase.

Continuing work with other antifolates led to the discovery that, like methotrexate, many antifolates undergo polyglutamylation. In 1988, Nair, Nanavati, Gaumont and Kisliuk reported in the Journal of Medicinal Chemistry that, like methotrexate, 10-deazaaminopterin and its 10-ethyl derivative undergo polyglutamylation (J. Med. Chem. 31:181, 1988) and these polyglutamyl derivatives inhibit the enzyme thymidylate synthase more effectively than the parent compound. The potent antileukemic agent $N^{10}$-propargyl-5,8-dideazafolic acid (CB 3717, PDDF) is converted to its polyglutamyl derivatives in normal murine tissues (M.G. Nair, Mehtha and I.G. Nair, Fed. Proc. 45:821, 1986). Nair, Nanavati, Kisliuk, Gaumont, Hsio and Kalman reported in the Journal of Medicinal Chemistry (29:1754, 1986) that the polyglutamyl derivatives of PDDF are more effective in inhibiting thymidylate synthetase.

Cheng, Dutschman, Starnes, Fisher, Nanavati and Nair in Cancer Research (34:598, 1985) and Ueda, Dutschman, Nair, DeGraw, Sirotnak and Cheng in Molecular Pharmacology (30:149, 1986) reported that the polyglutamyl derivatives of PDDF and 10-deazaaminopterin were more inhibitory to human thymidylate synthase than the non-polyglutamylated parent compounds. The antipurine effect of the well-known anticancer and anti-arthritic drug methotrexate has been attributed to its polyglutamyl

derivatives inhibiting the enzyme AICAR transformylase (Allegra, Drake, Jolivet and Chabner, Proc. Nat. Acad. Sci. USA 82:4881, 1985). These data taken together clearly show that the toxicity of classical antifolates like methotrexate, 10-deazaaminopterins and PDDF is potentiated by polyglutamylation. The accumulation of their toxic metabolites in normal human tissues such as kidneys, bone marrow and liver undoubtedly contributes to the undesirable side effects of these drugs and seriously undermines their clinical utility.

Therefore, it was of interest to us to develop classical antifolates that are powerful inhibitors of the target enzyme dihydrofolate reductase, but incapable of elaboration in vivo to their polyglutamyl derivatives. If these compounds are transported as efficiently as methotrexate to tumour cells, then such compounds should have clinical utility as anticancer drugs and exhibit lower host toxicity. Compounds 1, 2 and 3 all have shown excellent antitumour activity in three tumour models (Table I). All three compounds were similar to methotrexate in their efficacy in inhibiting the target enzyme dihydrofolate reductase (Table II). As shown in Table III, compounds 1 and 2 are transported more efficiently to H35 hepatoma cells than methotrexate as determined by their ability to compete with folinic acid transport in this cell line.

Compounds 1, 2 and 3 were evaluated as substrates and inhibitors of purified human leukaemia cell folylpolyglutamate synthetase (FPGS). It has been established that substrates of FPGS are capable of polyglutamylation in vivo and the relative magnitude of substrate activity of an antifolate to this enzyme compared to a standard is a measure of its relative ability to undergo polyglutamylation in vivo. In table IV, the relative substrate activities of compounds 1, 2 and 3 are presented compared to two standards, aminopterin and 10-deazaaminopterin. The data establish that the new compounds 1, 2 and 3 are not substrates of CCRF-CEM human leukaemia cell folylpolyglutamate synthetase.

Therefore, by analogy to methotrexate, compounds 1, 2 and 3 should have clinical utility as novel anticancer drugs capable of exhibiting lower host toxicity. In addition, they should be useful in the treatment of rheumatoid arthritis since they are expected to be immune suppressants to a similar degree as methotrexate.

This invention accordingly also provides a process for treating leukaemia, ascitic and solid tumours and rheumatoid arthritis which comprises administering to a warm-blooded animal with an abnormal proportion of leukocytes or other evidence of malignancy or rheumatoid arthritis a therapeutic nontoxic amount of compounds 1, 2 or 3 as such, or in the form of a pharmacologically acceptable salt thereof.

The process of the invention for the preparation of 4-amino-4-deoxy-10-deazapteroyl-γ-methyleneglutamic acid (1) and 4-amino-4-deoxy-10-ethyl-10-deazapteroyl-γ-methyleneglutamic acid (2) is a synthesis in which 4-amino-4-deoxy-10-deazapteroic acid (4) or 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid (5) prepared according to the procedure of Nair (Journal of Organic Chemistry 50:1879, 1985) is coupled with diethyl-γ-methylene glutamate (6) followed by base hydrolysis (Scheme 1 and Scheme 2). Likewise, the process of the invention for the preparation of 4-amino-4-deoxy-10-deazapteroyl-

3-hydroxyglutamic acid (3) is a synthesis in which 4-amino-4-deoxy-10-deazapteroic acid is coupled with dimethyl-3-hydroxyglutamate followed by base hydrolysis (Scheme 3).

Stage 1 (Schemes 1 or 2) is essentially the conversion of 4-amino-4-deoxy-10-deazapteroic acid (4) or 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid (5) to the corresponding mixed anhydrides 6 or 7 by treatment with an equal amount of aklylchloroformate such as isobutylchloroformate in an appropriate solvent such as dimethylformamide (DMF) in the presence of an acid acceptor, preferably a tertiary amine such as triethylamine or N-methylmorpholine. This reaction can be carried out within a temperature range of 0-30°C. Other acid acceptors such as substituted pyridines, tributylamine, collidine, lutidine or MgO may be substituted for triethylamine. The reaction may be conducted with other alkylchloroformates such as methyl, ethyl, propyl, etc. Other solvents such as dimethylsulfoxide, hexamethylphosphoramide or dimethylacetamide may be used for this reaction.

In stage II the mixed anhydride 6 or 7 is reacted with an excess amount of a diester of γ-methyleneglutamic acid such as diethyl-γ-methyleneglutamic acid. Dimethyl, dibenzyl or di-t-butyl-γ-methylene glutamic acid may be substituted for the diethyl derivative. Diethyl-γ-methylene glutamate may be added as the hydrochloride form to the mixed anhydride solutions 6 or 7, followed by the addition of an equal amount or an excess of an acid acceptor such as triethylamine. Alternatively, diethyl-γ-methyleneglutamate hydrochloride may be dissolved in a suitable solvent such as DMF, neutralised with an equivalent amount of the acid acceptor, and then added to the mixed anhydride solution. This reaction mixture is stirred for 18 hours at 25-30°C and the solvent is removed by evaporation under reduced pressure. The resulting coupled product is stirred with an excess of a mixture of 0.1 N NaOH and acetonitrile for 18 hours to give target compounds 1 or 2. The glutamic acid derivatives 1 and 2 are soluble in alkali and they can be isolated as precipitates by the addition of an acid to the basic hydrolysate. The precipitate can be recovered by filtration, washed and dried.

For the preparation of compound 3 (Scheme 3), the solution of the mixed anhydride 6 is reacted with an excess amount of the diester of 3-hydroxyglutamic acid such as dimethyl-3- hydroxyglutamate. Diethyl, dibenzyl or di-t-butyl-3-hydroxyglutamate may be substituted for the dimethyl derivative. Dimethyl-3-hydroxyglutamate may be added to the solution of the mixed anhydride as the hydrochloride followed by the addition of an equivalent or excess amount of the acid acceptor such as triethylamine. Alternatively, dimethyl-3-hydroxyglutamate hydrochloride may be dissolved in a suitable solvent such as DMF, neutralised with an equivalent amount of the acid acceptor such as triethylamine or N-methylmorpholine and then added to the mixed anhydride solution. The reaction mixture is then stirred for 18-24 hours at 25-30°C and the solvent is removed by rotary evaporation under reduced pressure. The resulting product is stirred with an excess of a 3:1 mixture of 0.1 N NaOH:acetonitrile for 10-18 hours to give target compound 3. Compound 3 is soluble in alkali and it can be isolated as a precipitate by addition of an acid such as glacial acetic acid to the hydrolysate. The precipitate can be recovered by filtration, washed and dried.

The following examples illustrate application of the synthesis to the preparation of γ-methyleneglutamic acid derivatives 1 and 2, and 3-hydroxyglutamic acid derivative 3.

## Example I

### Diethyl-γ-methyleneglutamate hydrochloride

γ-Methyleneglutamic acid was purchased from CalBiochem- Behring. In a round bottomed flask, 795 mg (5 mmol) of the above γ-methyleneglutamic acid was dissolved in 25 mL of absolute ethyl alcohol. The solution was cooled to 0°C in an ice bath, and 3 mL of thionyl chloride was slowly added while stirring. After the addition was complete, the reaction mixture was allowed to stir for 18 hours at 25°C, then refluxed for four hours and evaporated until a semi solid was obtained. Upon trituration with 40 mL of diethyl ether, diethyl-γ-methyleneglutamate crystallised as a white solid: mp 87-89°C; mass spectrum (FAB) m/z, 217 ($MH^+$); yield 1.0 g (80%).

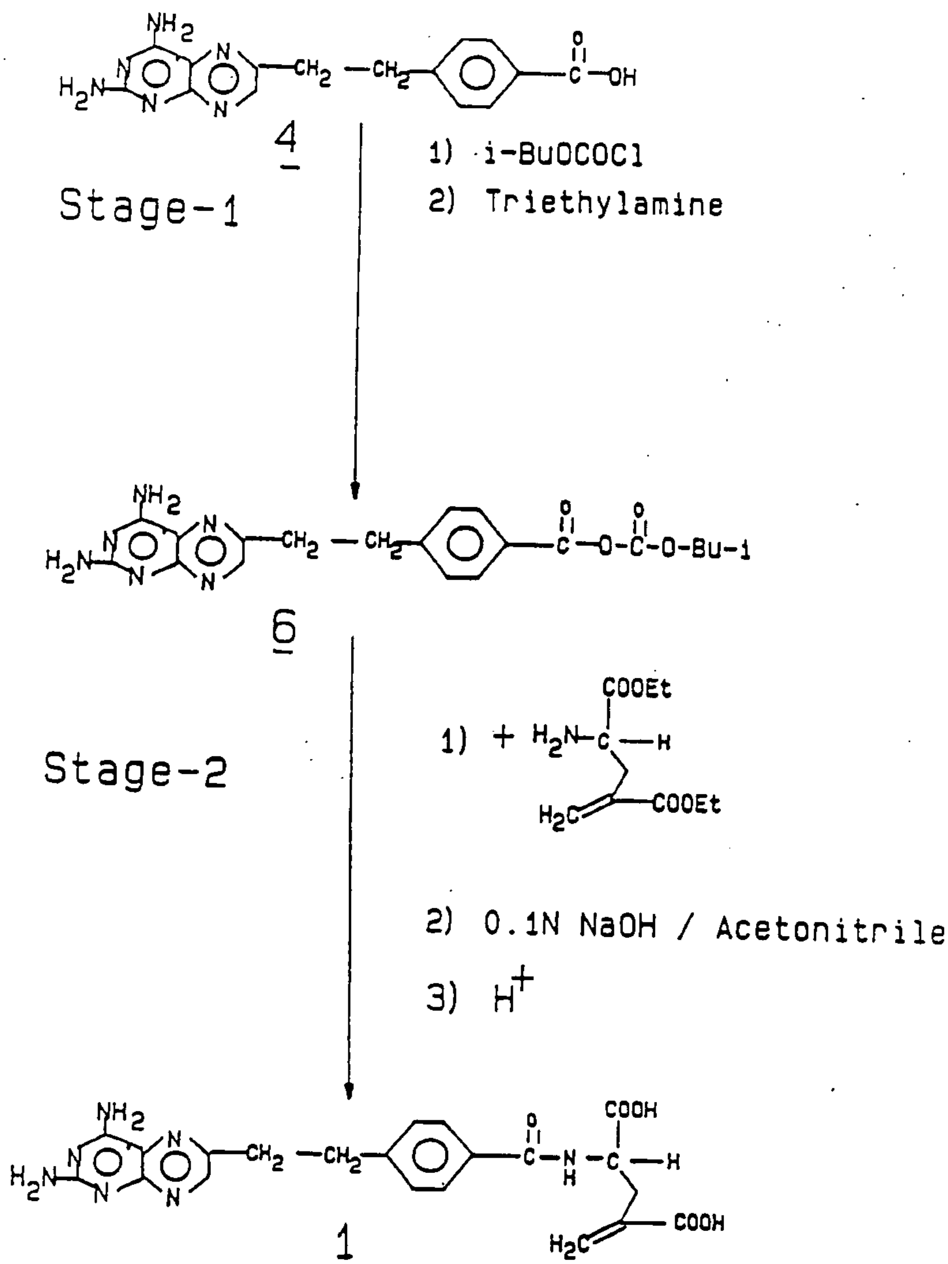
### 4-Amino-4-deoxy-10-deazapteroyl-γ-methyleneglutamic acid (1)

A solution of 310 mg (1 mmol) of 4-amino-4-deoxy-10-deazapteroic acid (prepared according to the procedure of Nair as described in J. Org. Chem. 50:1879, 1985) in 40 mL of dry DMF was prepared by the addition of 0.28 mL (2 mmol) of triethylamine and warming the mixture to 80-90°C. The solution was then cooled to ~5°C with the aid of an ice bath, and 0.262 mL (2 mmol) of freshly distilled isobutylchloroformate was added while stirring. After 15 minutes, the ice bath was removed and there action mixture was allowed to warm to 25°C and kept for 30 minutes to complete the formation of the mixed anhydride 6. To this mixed anhydride solution was added 503 mg (2 mmol) of diethyl-γ-methyleneglutamate hydrochloride immediately followed by the addition of 0.28 mL (2 mmol) of triethylamine. The resultant reaction mixture was stirred for 18 hours at 25-30°C, evaporated under reduced pressure at 70°C, triturated with 50 g of crushed ice, and filtered. The precipitate was stirred with a mixture of 50 mL of 0.1 N NaOH and 15 mL of acetonitrile for 18 hours, when a clear solution was obtained. The pH of this solution was adjusted to 7.5 with 1 N HCl, concentrated by rotary evaporation to ~20 mL, cooled and acidified with glacial acetic acid to pH 4.0. The resulting yellow precipitate was filtered, washed and dried. HPLC analysis established that the product was a mixture of unreacted 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid and the desired 4-amino-4-deoxy-10-deazapteroyl-γ-methylene glutamic acid (1) in a ratio of 1:3.

The target compound 1 was separated from this mixture by dissolving the crude product (400 mg) in 10 mL of 5% ammonium hydroxide solution and evaporating the solvent by rotary evaporation under reduced pressure. The ammonium salt thus obtained was dissolved in 10 mL of distilled water and applied on a column of 15 g of C18 silica gel equilibrated with 10% acetonitrile in water. The column was eluted with 10% acetonitrile in water and 5 mL fractions were collected. Fractions, corresponding to the fast moving band on the column were pooled and acidified with glacial acetic acid to obtain a

# Scheme-1

## Steps of the reaction synthesis of 4-amino-4-deoxy-10-deazapteroyl-γ-methyleneglutamic acid (1)



bright yellow precipitate which was filtered, washed with distilled water and dried: yield, 300 mg (67%); mp >300°C. This compound gave the following analytical data consistent with the structure.

| Mol formula | Mol wt (calcd) | (found) | 0.1 N NaOH λmax nm (ε) |
|---|---|---|---|
| $C_{21}H_{21}N_7O_5$ | 451 | 451 | 254 (30,704)<br>369 ( 6,859) |

## Example II

Diethyl-γ-methyleneglutamate hydrochloride

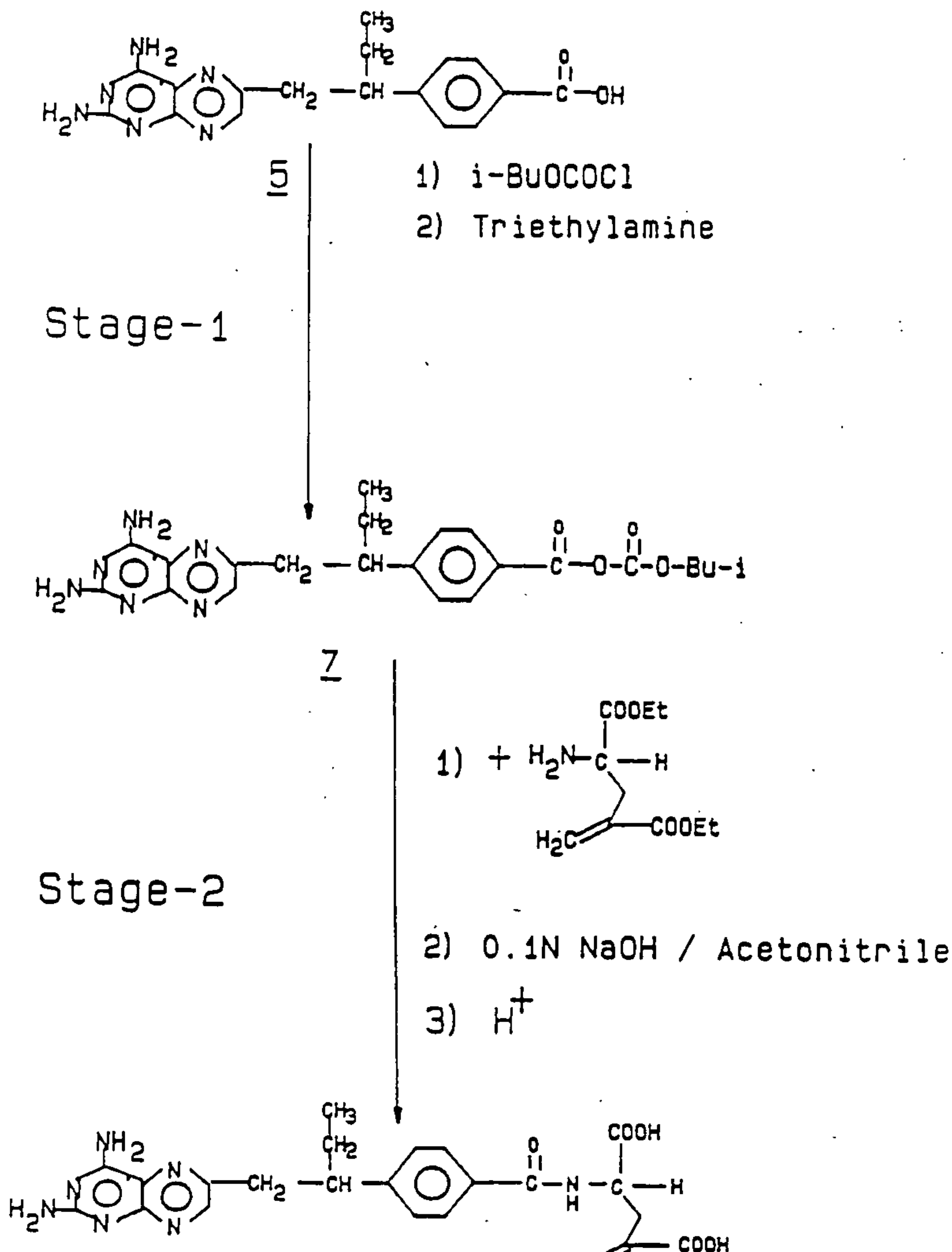
γ-methyleneglutamic acid was purchased from CalBiochem-Behring. In a round bottomed flask, 795 mg (5 mmol) of the above γ-methylene-glutamic acid was dissolved in 25 mL of absolute ethyl alcohol. The solution was cooled to 0°C in an ice bath, and 3 mL of thionyl chloride was slowly added while stirring. After the addition was complete, the reaction mixture was allowed to stir for 18 hours at 25°C, then refluxed for four hours and evaporated until semi solid. Upon trituration with 40 mL of diethyl ether, diethyl-γ-methyleneglutamate crystallised as a white solid: mp 87- 89°C; mass spectrum (FAB) m/z, 216 ($MH^+$); yield 1.0 g (80%).

4-Amino-4-deoxy-10-ethyl-10-deazapteroyl-γ-methyleneglutamic acid (2)

A solution of 337 mg (1 mmol) of 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid (prepared according to the procedure of Nair as described in J. Org. Chem. 50:1879, 1985) in 40 mL of dry DMF was prepared by the addition of 0.226 mL (2 mmol) of N-methylmorpholine and warming the mixture to 80-90°C. The solution was then cooled to ~5°C with the aid of an ice bath, and 0.262 mL (2 mmol) of freshly distilled isobutylchloroformate was added while stirring. After 15 minutes, the ice bath was removed and the reaction mixture was allowed to warm to 25°C and kept for 30 minutes to complete the formation of the mixed anhydride 7. To this mixed anhydride solution was added 503 mg (2 mmol) of diethyl-γ-methyleneglutamate hydrochloride immediately followed by the addition of 0.226 mL (2 mmol) of N-methylmorpholine. The resultant reaction mixture was stirred for 18 hours at 25-30°C, evaporated under reduced pressure at 70°C, triturated with 50 g of crushed ice, and filtered. The precipitate was stirred with a mixture of 50 mL of 0.1 N NaOH and 15 mL of acetonitrile for 18 hours, when a clear solution was obtained. The pH of this solution was adjusted to 7.5 with 1 N HCl, concentrated by rotary evaporation to ~20 mL, cooled and acidified with glacial acetic acid to pH 4.0. The resulting yellow precipitate was filtered, washed and dried. HPLC analysis established that the product was a mixture of

# Scheme-2

Steps of the reaction synthesis of 4-amino-4-deoxy -10-ethyl-10-deazapteroyl-γ-methyleneglutamic acid (2).




2

unreacted 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid and the desired 4-amino-4-deoxy-10-ethyl-10-deazapteroyl-γ-methylene glutamic acid (2) in a ratio of 1:3.

The target compound 2 was separated from this mixture by dissolving the crude product (420 mg) in 10 mL of 5% ammonium hydroxide solution and evaporating the solvent by rotary evaporation under reduced pressure. The ammonium salt thus obtained was dissolved in 10 mL of distilled water and applied on a column of 15 g of C18 silica gel equilibrated with 10% acetonitrile in water. The column was eluted with 10% acetonitrile in water and 5 mL fractions were collected. Fractions, corresponding to the fast moving band on the column were pooled and acidified with glacial acetic acid to obtain a bright yellow precipitate which was filtered, washed with distilled water and dried: yield, 350 mg (~69%); mp >300°C. This compound gave the following analytical data:

| Mol formula | Mol wt (calcd) | (found) | 0.1 N NaOH λmax nm (ε) |
|---|---|---|---|
| $C_{23}H_{25}N_7O_5$ | 479 | 479 | 253 (31,806)<br>370 ( 7,491) |

## Example III

### Dimethyl-3-hydroxyglutamate hydrochloride

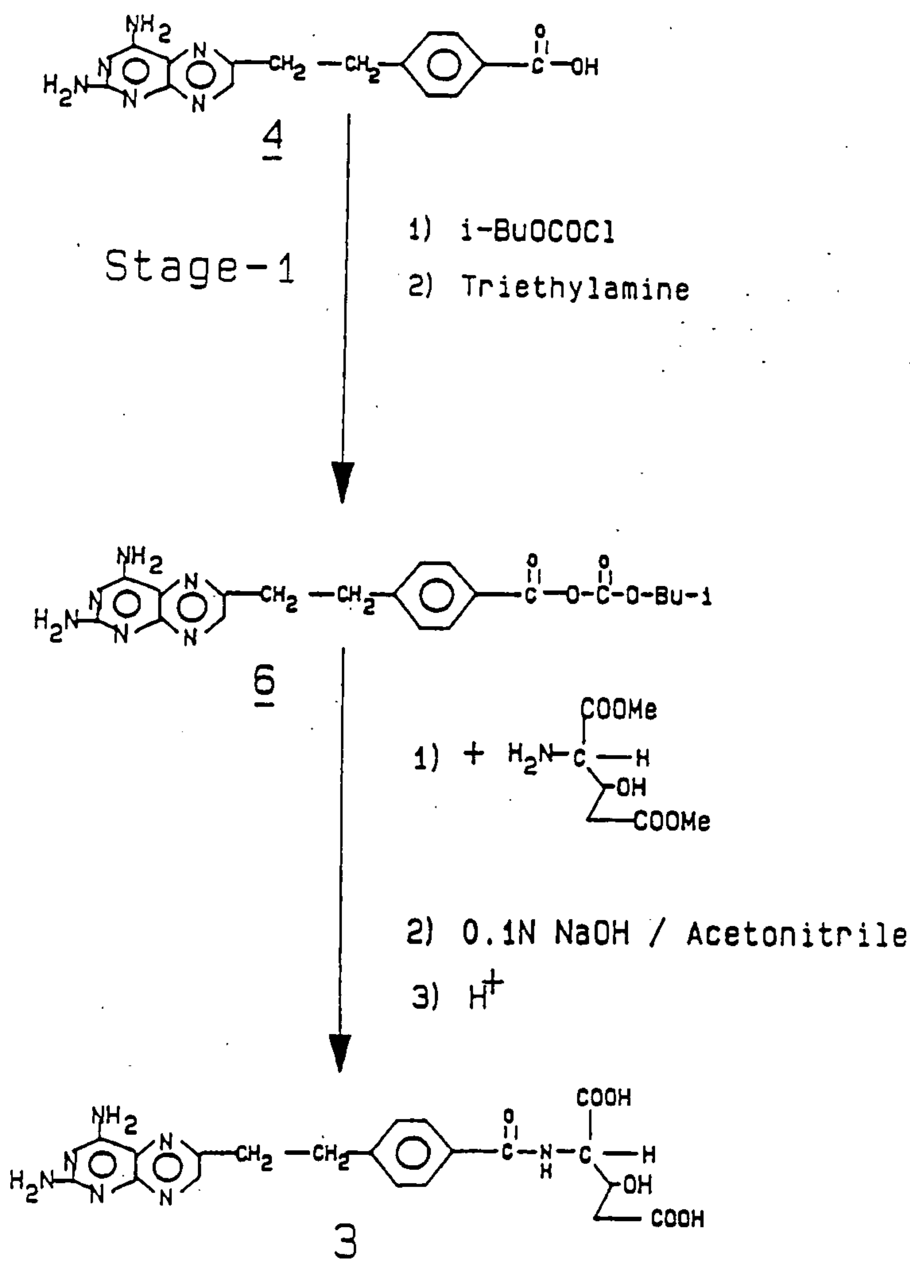
3-Hydroxyglutamic acid was prepared according to the procedure of Vidal-Cros, Gaudry and Marquet as described in the Journal of Organic Chemistry 50:3163 (1985). In a round-bottomed flask 8.0 g (~50 mmol) of powdered 3-hydroxyglutamic acid was suspended in 250 mL of dry methanol and the mixture cooled to 0°C with the aid of an ice bath. Thionylchloride (25 mL) was slowly added to the solution and when the addition was complete, the ice bath was removed and the solution was allowed to stir for 18 hours at 25°C. It was then refluxed for four hours, 200 mL of benzene was added and evaporated under reduced pressure to dryness. The resultant residue was dissolved in 100 mL of acetone and with the slow addition of diethyl ether and trituration crystals were formed. These crystals were separated by filtration, washed with 50 mL of ether followed by 15 mL of acetone and dried in a vacuum over $P_2O_5$: yield 58%; mp 137-138°C; NMR (TFA) δ4.75 (br, d, 1H, αH), 3.79, 3.65 (s, s, 3H, 3H, carbomethoxy), 2.82 (d, 2H, $-CH_2-$); mass spectrum, calculated for $C_7H_{13}NO_4$, ($MH^+$) 192, found 192.

### 4-Amino-4-deoxy-10-deazapteroyl-3-hydroxyglutamic acid (3)

Exactly one mmole (310 mg) of the 4-amino-4-deoxy-10-deazapteroic acid was dissolved in 100

## Scheme-3

**Steps in the reaction synthesis of 4-amino-4-deoxy-10-deazapteroyl-3-hydroxyglutamic acid (3).**



mL of DMF by heating to 80°C; 0.28 mL (2 mmol) of freshly distilled triethylamine was added and the solution cooled to 0°C. Then 0.262 mL (2 mmol) isobutylchloroformate was added and the reaction mixture was stirred for 90 minutes at 0-5°C. The reaction mixture containing the mixed anhydride (6) was removed from the ice bath and kept stirring for 30 minutes at 25°C. Dimethyl-3- hydroxyglutamate hydrochloride (456 mg; 2 mmol) was dissolved in 15 mL of DMF and 0.28 mL (2 mmol) freshly distilled triethylamine was added to this solution. This was then added to the mixed anhydride solution and the resulting mixture stirred for 18 hours at 25°C, heated to 55°C and evaporated to dryness in a vacuum. $NaHCO_3$ (10 mL of 5%) and 10 mL of ether was added to the residue and stirred for one hour. The solution was filtered and the precipitate washed with water. The dimethylester precipitate was suspended in 80 mL of 0.1 N NaOH and 25 mL of acetonitrile was added to obtain a clear solution. The solution was stirred for 18 hours at 25°C. The acetonitrile was removed under vacuum, the pH of the solution adjusted to 7.5-8 with 1 N HCl and the product purified by ion exchange chromatography over DEAE cellulose in the chloride form using a linear NaCl gradient from 0-0.5 M. The product was precipitated by acidifying the pooled fractions corresponding to the product to pH 3.5 with glacial acetic acid. The solution was chilled and filtered. The precipitate of 3 was washed with water and dried: yield, 56%.

4-Amino-4-deoxy-10-deazapteroyl-3-hydroxyglutamic acid (3) analyzed as follows:

| Mol formula | Mol wt (calcd) | (found) | 0.1 N NaOH λmax nm (ε) |
|---|---|---|---|
| $C_{20}H_{21}N_7O_6$ | 455 | 455 | 256 (22,870)<br>370 ( 7,886) |

The glutamate compounds 1, 2 or 3 can be used as such or in the form of salts that are formed with one or more amino groups of the pteridine ring. Acids such as hydrochloric, hydrobromic, sulfonic, nitric, phosphoric, and organic carboxylic acids such as maleic, citric, salicylic and methane sulfonic acid may be used for the preparation of the addition salts.

The glutamate compounds 1, 2 or 3 or salts thereof may be administered to a warm-blooded animal by oral and parenteral (intraperitoneal, intravenous, intrathecal, subcutaneous, intramuscular) administration. A dosage of 1, 2 or 3 in the amount of 0.1 mg to about 500 mg/kg to ameliorate the leukaemia, ascites or solid tumours or rheumatoid arthritis in humans will be sufficient. The higher dosage amount of about 500 mg/kg may be administered in conjunction with leucovorin [6(RS) 5-formyltetrahydrofolate] to further reduce toxicity.

The glutamate compounds 1, 2 or 3 can be provided in composite forms to facilitate administration or in dosage unit form. A sterile and nontoxic carrier may be added to 1, 2 or 3; the carrier may be a liquid, solid, or semisolid which may serve as a vehicle, medium or excipient. The

carriers may include gelatin, methylcellulose, propylhydroxybenzoate, talc, magnesium stearate, oil of theobroma, gum aracia, lactose, dextrose, mannitol, sorbitol and mineral oil. The glutamate compounds 1, 2 or 3 and carrier or diluent can be enclosed or encapsulated in a paper or other container, capsule, cachet, gelatin, or sachet when intended for use in dosage units. The dosage units can take the form of cachets, suppositories, capsules or tablets.

TABLE I

INHIBITION OF TUMOUR CELL GROWTH BY 1, 3 AND 3

| Compound | $I_{50}$ (nM) H35 hepatoma | Human manca lymphoid leukaemia | CCRF-CEM human leukaemia |
|---|---|---|---|
| 1 | 9.0 | 7.0 | 5.0*<br>4.0 |
| 2 | 11.0 | 9.0 | 6.0*<br>4.0 |
| 3 | 28.0 | 15.0 | --- |
| Methotrexate | 10.0 | 6.0 | 12.0<br>8.0 |

Manca cell growth assay was performed as described by Thorndike, Gaumont, Kisliuk, Sirotnak, Murthy and Nair in Cancer Research 49:158 (1989). Growth inhibition of H35-hepatoma cells was measured as described by Patil, Jones, Nair, Galivan, Maley, Kisliuk, Gaumont, Duch and Ferone in the Journal of Medicinal Chemistry 32:1284 (1989) and that of CCRF-CEM human leukaemia cells according to the procedures of McGuire, Graber, Licato, Vincenz, Coward, Nimec and Galivan (Cancer Research 49:4517, 1989).

*Corrected for the presence of the d-enantiomer of γ-methyleneglutamate. The values correspond to duplicate experiments.

## TABLE II

INHIBITION OF DIHYDROFOLATE REDUCTASE BY 1, 2, AND 3

| Compound | $IC_{50}$ (nM) |
|---|---|
| 1 | 30.00 |
| 2 | 32.00 |
| 3 | 33.00 |
| Methotrexate | 22.00 |

Pure human dihydrofolate reductase from lymphoblastoid cells were used. The enzymatic assay was performed as described by Pastore, Plante and Kisliuk in Methods in Enzymology 34:281 (1974).

## TABLE III

INHIBITION OF FOLINIC ACID TRANSPORT TO H35 HEPATOMA CELLS

| Compound | $I_{50}$ (nM) |
|---|---|
| Methotrexate | 18.00 |
| 1 | 4.00 |
| 2 | 3.00 |

Transport experiments were conducted as described by Patil, Jones, Nair, Galivan, Maley, Kisliuk, Gaumont, Duch and Ferone in the Journal of Medicinal Chemistry 32:1284 (1989). Folinic acid (2 $\mu$M, 15 min uptake.)

TABLE IV

SUBSTRATE ACTIVITY OF 4-AMINO-4-DEOXY-10-DEAZAPTEROYL-γ-METHYLENEGLUTAMIC ACID (1), 4-AMINO-4-DEOXY-10-ETHYL-10-DEAZAPTEROYL-γ-METHYLENEGLUTAMIC ACID (2) AND 4-AMINO-4-DEOXY-10-DEAZAPTEROYL-3-HYDROXYGLUTAMIC ACID (3) TOWARDS CCFR-CEM HUMAN LEUKAEMIA CELL FOLYLPOLYGLUTAMATE SYNTHETASE (FPGS)

| COMPOUND | Conc (μM) | Substrate activity |
|---|---|---|
| 1 | 50 | 5 |
| | | 3 |
| 2 | .50 | 3 |
| | | 6 |
| 3 | 50 | 8 |
| | | 10 |
| Controls | | |
| Aminopterin | 50 | 950 |
| 10-Deazaaminopterin | 50 | 1020 |

The assays were performed as described by McGuire, Graber, Licato, Vincenz, Coward, Nimec and Galivan in Cancer Research 49:4517 (1989).

The process of this invention is illustrated by the reaction sequence depicted in Schemes 1, 2 and 3 where the compound numbers identify the same compounds which they identify in all descriptions.

## USE ADVANTAGE

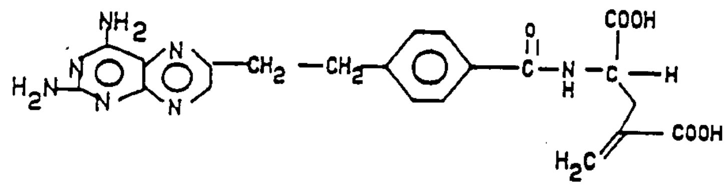
The most widely used antifolate drug for the treatment of human cancers is methotrexate (MTX). Recently 10-ethyl-10-deazaaminopterin (10-EDAM), which is an analogue of methotrexate, has been shown to exhibit a wide spectrum of antitumour activity. Both MTX and 10-EDAM are metabolized to their poly-$\gamma$-glutamyl derivatives. The target enzyme for both antifolates (MTX and 10-EDAM) is dihydrofolate reductase. 10-EDAM has the additional advantage of enhanced transport to mammalian cells compared to MTX. The polyglutamyl metabolites of MTX and 10-EDAM inhibit other folate-based enzymes in addition to dihydrofolate reductase. Therefore, antifolate polyglutamylation results in the potentiation of cytotoxicity and undoubtedly contributes to the undesirable toxic side effects of the drugs because of the accumulation of these toxic metabolites and their prolonged retention in normal proliferative tissues such as liver, kidney, intestinal epithelium and bone marrow.

Any classical antifolate that has biochemical properties identical to methotrexate (such as dihydrofolate reductase inhibition and transport to tumour cells), but which is incapable of polyglutamylation should show remarkably less host toxicity. Compounds 1, 2 and 3 show dihydrofolate reductase inhibition similar to that of methotrexate. Antifolates 1 and 2 are transported to tumour cells three to five times more efficiently than methotrexate. On a comparative basis, 1 and 2 are twice as powerful as methotrexate in inhibiting the growth of human leukaemia cells. None of the new antifolates (1, 2 or 3) is a substrate for folylpolyglutamate synthetase and therefore cannot be metabolised to poly-$\gamma$-glutamates. In accordance with the invention it was determined that compounds 1 ,2 and 3 are new and novel non-polyglutamatable and relatively nontoxic antifolates that should be useful in the treatment of human cancers and they are immune suppressants with activity similar to or better than methotrexate. The dose is 0.1-500 mg/kg daily in humans orally or parenterally.

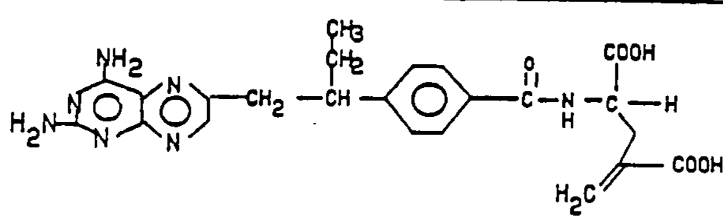
Having regard to the foregoing disclosure, the following is claimed as inventive and patentable embodiments thereof

We Claim:

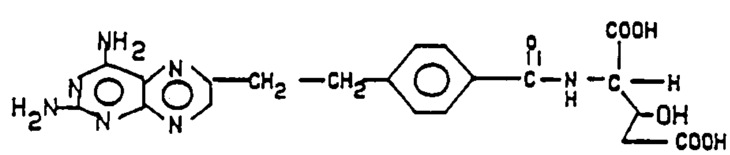
1. 4-Amino-4-deoxy-10-deazapteroyl-γ-methylene-glutamic acid having the formula:



2. 4-Amino-4-deoxy-10-ethyl-10-deazapteroyl-γ-methyleneglutamic acid having the formula:



3. 4-Amino-4-deoxy-10-deazapteroyl-3-hydroxy-glutamic acid having the formula:



4. A pharmaceutical composition in dosage unit form for treating leukaemia, ascites tumours or solid tumours comprising an amount within the range of about 0.1 to about 500 mg of 4-amino-4-deoxy-10-deazapteroyl-$\gamma$-methyleneglutamic acid per dosage unit therapeutically effective to ameliorate leukaemia, ascites tumours or solid tumours together with a pharmaceutically acceptable nontoxic carrier or diluent thereof.

5. A pharmaceutical composition in dosage unit form for treating leukaemia, ascites tumours or solid tumours comprising an amount within the range of about 0.1 to about 500 mg of 4-amino-4-deoxy-10-ethyl-10-deazapteroyl-$\gamma$-methyleneglutamic acid per dosage unit therapeutically effective to ameliorate leukaemia, ascites tumours or solid tumours together with a pharmaceutically acceptable nontoxic carrier or diluent thereof.

6. A pharmaceutical composition in dosage unit form for treating leukaemia, ascites tumours or solid tumours comprising an amount within the range of about 0.1 to about 500 mg of 4-amino-4-deoxy-10-deazapteroyl-3-hydroxy-glutamic acid per dosage unit therapeutically effective to ameliorate leukaemia, ascites tumours or solid tumours together with a pharmaceutically acceptable nontoxic carrier or diluent thereof.

7. A process for the treatment of rheumatoid arthritis which comprises admininstering to a warm-blooded animal having rheumatoid arthritis a therapeutic amount of 4-amino-4-deoxy-10-deazapteroyl-$\gamma$-methyleneglutamic acid to ameliorate the rheumatoid arthritis.

8. A process for the treatment of rheumatoid arthritis which comprises admininstering to a warm-blooded animal having rheumatoid arthritis a therapeutic amount of 4-amino-4-deoxy-10-ethyl-10-deazapteroyl-$\gamma$-methyleneglutamic acid to ameliorate the rheumatoid arthritis.

9. A process for the treatment of rheumatoid arthritis which comprises admininstering a therapeutic amount of 4-amino-4-deoxy-10-deazapteroyl-3-hydroxyglutamic acid to ameliorate the rheumatoid arthritis.

10. A process for preparing 4-amino-4-deoxy-10-deazapteroyl-$\gamma$-methyleneglutamic acid according to Claim 1, which comprises of converting the known 4-amino-4-deoxy-10-deazapteroic acid to a mixed anhydride by reaction with an alkylchloroformate, reacting the resulting mixed anhydride with a diester of $\gamma$-methyleneglutamic followed by base hydrolysis and acidification.

11. A process for preparing 4-amino-4-deoxy-10-ethyl-10-deazapteroyl-$\gamma$-methyleneglutamic acid according to Claim 2, which comprises of converting the known 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid to a mixed anhydride by reaction with an alkylchloroformate, reacting the resulting mixed anhydride with a diester of $\gamma$-methyleneglutamic acid followed by base hydrolysis and acidification.

12. A process for preparing 4-amino-4-deoxy-10-deazapteroyl-3-hydroxyglutamic acid according to Claim 3, which comprises of converting the known 4-amino-4-deoxy-10-deazapteroic acid to a mixed anhydride by reaction with an alkylchloroformate, reacting the resulting mixed anhydride with a diester of 3-hydroxyglutamic acid followed by base hydroylsis and acidification.

13. A process for treating leukaemia, ascites tumours or solid tumours which comprises admininstering orally or parenterally to a warm-blooded animal having an abnormal proportion of leukocytes or other evidence of malignancy, a therapeutic and relatively nontoxic amount of 4-amino-4-deoxy-10-deazapteroyl-γ-methyleneglutamic acid to ameliorate leukaemia, ascites tumours or solid tumours.

14. A process for treating leukaemia, ascites tumours or solid tumours which comprises administering orally or parenterally to a warm-blooded animal having an abnormal proportion of leukocytes or other evidence of malignacy, a therapeutic and relatively nontoxic amount of 4-amino-4-deoxy-10-ethyl-10-deazapteroyl-γ-methyleneglutamic acid to ameliorate leukaemia, solid or ascites tumours.

15. A process for treating leukaemia, ascites tumours or solid tumours which comprises administering orally or parenterally to a warm-blooded animal having an abnormal proportion of leukocytes or other evidence of malignacy, a therapeutic and relatively nontoxic amount of 4-amino-4-deoxy-10-deazapteroyl-3-hydroxyglutamic acid to ameliorate leukaemia, ascites tumours or solid tumours.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/00220

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07D 475/08, A61K 31/505 U.S. Cl. 544/260

**II. FIELDS SEARCHED**

Minimum Documentation Searched [4]

| Classification System | Classification Symbols |
|---|---|
| US | 544/260<br>514/249,258 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CAS online Structure Search

**III. DOCUMENTS CONSIDERED TO BE RELEVANT** [14]

| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No. [18] |
|---|---|---|
| X | Jour. Organic Chem. Vol. 50, No. 11 1985, M. G. Nair, "Folate Analogues. 24, pages 1879-1884, see paragraph bridging pages 1883-4. | 10-12 |
| Y | US, A, 4,532,241 (DeGRAW) 30 July 1985, See Col. 1, lines 30-36. | 3,6,9,15 |
| Y | JP, B, 0081385 (SHIONOGI) 14 April 1987, See page 5 compound 8. | 3,6,9,15 |
| E | US, A,4,996,207 (NAIR) 18 January 1990. | |
| X | US, A, 4,369,319 (DeGraw) 18 January 1983, See Columns 3-4, "Stage 7" to "State 8". | 10-12 |
| A | Jour. Med. Chem. Vol. 17, No. 5, 1974, DeGraw "Synthesis and Activity of 10-Deazaminopterin", pp. 552-3. | |
| A | Jour. Med. Chem., Vol. 17, No. 3, 1974, Lee "Folic Acid Antagonists", pp. 326-330. | |

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search [2] | Date of Mailing of this International Search Report [2] |
|---|---|
| 27 FEBRUARY 1991 | 16 APR 1991 |
| **International Searching Authority [1]** | **Signature of Authorized Officer [20]** |
| ISA/US | D. G. DAUS |

Form PCT/ISA/210 (second sheet) (May 1986)